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Review

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Activation of membrane-bound proteins and receptor systems: a link between tissue kallikrein and the KLK-related peptidases

Abstract: The 15 members of the kallikrein-related serine peptidase (KLK) family have diverse tissue-specific expression profiles and roles in a range of cellular processes, including proliferation, migration, invasion, differentiation, inflammation and angiogenesis that are required in both normal physiology as well as pathological conditions. These roles require cleavage of a range of substrates, including extracellular matrix proteins, growth factors, cytokines as well as other proteinases. In addition, it has been clear since the earliest days of KLK research that cleavage of cell surface substrates is also essential in a range of KLK-mediated cellular processes where these peptidases are essentially acting as agonists and antagonists. In this review we focus on these KLK-regulated cell surface receptor systems including bradykinin receptors, proteinase-activated receptors, as well as the plasminogen activator, ephrins and their receptors, and hepatocyte growth factor/Met receptor systems and other plasma membrane proteins. From this analysis it is clear that in many physiological and pathological settings KLKs have the potential to regulate multiple receptor systems simultaneously; an important issue when these peptidases and substrates are targeted in disease.

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Introduction

Tissue kallikrein or kallikrein 1 (*KLK1*/KLK1) was the first described member of a gene family located at human chromosome 19q13.4 that encodes 15 serine proteinases named kallikrein-related peptidases (KLKs) (Gan et al., 2000; Harvey et al., 2000; Yousef and Diamandis, 2001). The action of KLK1, the archetypal member of this family, was first recognised over 100 years ago in the work of Abelous and Bardier who discovered that an alcohol-insoluble fraction of human urine caused peripheral vasodilation and hypotension in dogs (Abelous and Bardier, 1909). Later studies showed that the hypotensive effect occurs, at least in part, by the action on bradykinin receptors, a sub-family of G protein-coupled receptors, of kinin peptides released from kininogens by KLK1 (Werle, 1934; Rocha e Silva et al., 1949; Bhoola et al., 1992). Notably, it became clear from the earliest days of research on the extended KLK family that these KLK1-related secreted extracellular peptidases also can have important physiological and pathological effects via actions on cell surface proteins. As summarised in Figure 1 and Table 1, it is now clear that these effects can occur both indirectly, for example by processing of precursor ligands as is the case of KLK1 cleavage of kininogens; and directly, for example by proteolysis of cell surface proteins as has been reported also to occur when bradykinin receptors are cleaved by KLK1. This theme has certainly been borne out for other members of the KLK family with many examples reported over the last 20 years

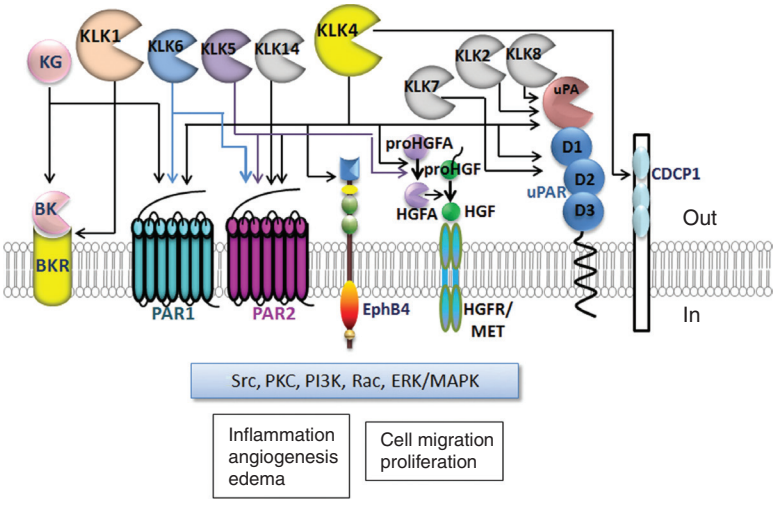

Figure 1 Schematic diagram showing KLKs 1, 2 and 4-8 activatation of receptors and/or membrane-bound factors initiating their downstream signalling pathways.
BK, bradykinin; BKR, bradykinin receptors 1 and 2; CDCP1, CUB domain containing protein; HGF(A), hepatocyte growth factor (activator); KG, kininogen; KLK1, tissue kallikrein; PAR, proteinase-activated receptor; uPA(R), urokinase plasminogen activator (receptor).

of KLK-mediated phenotypes that require activation of cell surface receptor systems. In the following sections we summarise these findings with a particular focus on bradykinin receptors, proteinase-activated receptors, as well as the plasminogen activator and hepatocyte growth factor/Met receptor systems and other cell surface proteins.

Kallikrein-kinin system

More than 100 years ago, Abelous and Bardier began to elucidate an important signalling pathway that has come to be known as the kallikrein-kinin system. The activating enzymes for this system are plasma kallikrein and tissue kallikrein, which are derived from two unrelated genes (Clements, 2000). The focus of this review is tissue kallikrein or KLK1. Tissue kallikrein (KLK1), the founding member of the kallikrein-related peptidase (KLK) family (Kraut et al., 1930), is a glycoprotein with trypsin-like substrate specificity for cleavage after arginine (Arg) and lysine (Lys) residues. It is expressed in a range of tissues and organs, and can process kininogens, growth factors, and extracellular matrix (ECM) molecules to regulate inflammation and pain, blood vessel dilation and permeability, blood pressure and sodium homeostasis control, kidney, heart and neurological function (Fiedler, 1979; Schachter, 1979; Brady et al., 1989; Clements, 2000; Chao et al., 2006; Madeddu et al., 2007; Savvatis et al., 2010; Rhaleb et al., 2011; Stadnicki, 2011; Viel and Buck, 2011; Kayashima et al., 2012; Regoli et al., 2012; da Costa et al., 2014). As an extracellular peptidase, KLK1 exerts these pleotropic biological effects by cleavage of the substrate, low molecular weight (LMW) kininogen, generating the kinin, lys-bradykinin, which exerts its biological functions via binding to the G protein-coupled receptor, the bradykinin B2 (Marcondes and Antunes, 2005). The related bradykinin B1 receptor is predominantly activated by a cleaved degradation product of lys-bradykinin at its C-terminal Arg by kininase I, lys-des-arg-bradykinin. The B1 receptor is largely an inducible receptor up-regulated in inflammatory conditions and tissue injury; conversely, the B2 receptor is constitutively expressed in a diverse range of healthy tissues mediating the action of kinins as well as up-regulated in disease (Madeddu et al., 2007; Savvatis et al., 2010; Stadnicki 2011; Viel and Buck, 2011; Kayashima et al., 2012; Regoli et al., 2012). KLK1 also directly induces rat uterine contraction and cardio-protection independent of kinin formation (Chao et al., 1981, 2008), further suggesting KLK1 has potential biological functions by acting on other substrates in addition to kininogen. A recent study showed that migration of circulating proangiogenic cells induced by KLK1 may not be all mediated by kinin *in vitro* while kinins are involved in the function of KLK1 *in vivo* (Spinetti et al., 2011). Of particular interest to this review, the human bradykinin B2 receptor can be activated in kininogen-free systems in which cells are exposed to KLK1 and trypsin, but the direct cleavage of the B2 receptor to generate a signal has yet to be observed (Hecquet et al., 2000; Biyashev et al., 2006). It thus remains to be seen if the B2 receptor can be considered as a 'proteolytically-activated receptor' akin to the proteinase-activated receptors (PARs), which are

Table 1 Summary of activation of cell membrane proteins and receptors by KLKs.

Kallikreins	Membrane proteins/receptors and signalling systems	References
KLK1	Kininogen: KLK1-(brady)kinin system	(Marcondes and Antunes, 2005)
	(Brady)kinin receptor (BKR)2	(Biyashev et al., 2006)
	PAR1: PAR1-PKC-Src-MMP, keratinocyte migration;	(Gao et al., 2010a)
	migration of prostate cancer cells	(Gao et al., 2010b)
	BKR2, migration of circulating proangiogenic cells	(Spinetti et al., 2011)
KLK2	uPA	(Frenette et al., 1997)
KLK4	PAR1, PAR2,	(Mize et al., 2008; Ramsay et al., 2008; Gratio et al., 2010)
	uPA-uPAR	(Takayama et al., 2001; Beaufort et al., 2006)
	pro-meprin β	(Becker-Pauly et al., 2007; Ohler et al., 2010)
	EphB4	(This review)
	HGF/MET	(Mukai et al., 2008)
	CDCP1	This review
KLK5	Pro-HGFA	(Mukai et al., 2008)
	PAR2	(Stefansson et al., 2008)
	Desmoglein 1, cell-cell junction in oral squamous cancer	(Jiang et al., 2011)
KLK6	Amyloid precursor protein (APP) in Alzheimer's disease and Parkinson's disease;	(Little et al., 1997; Ogawa et al., 2000; Magklara et al., 2003)
	PAR1, PAR2, PAR4	(Oikonomopoulou et al., 2006)
KLK7	Desmocollin, corneodesmosin;	(Caubet et al., 2004)
	E-cadherin	(Johnson et al., 2007)
	uPAR	(Ramani and Haun, 2008a)
KLK8	rPAR2 but not hPAR2	(Stefansson et al., 2008; Ramachandran et al., 2012)
KLK14	PAR2	(Stefansson et al., 2008; Gratio et al., 2011; Chung et al., 2012)

activated by a proteolytically-unmasked tethered ligand (Adams et al., 2011). These data have for the first time shown that a KLK peptidase could activate a membrane-bound receptor and led the way for many of the studies described below.

Proteinase-activated receptors

PARs are G protein-coupled receptors – PAR1 to PAR4 – that are activated by proteolysis primarily mediated by serine proteinases with trypsin-like specificity for cleavage after Arg and Lys, including members of the KLK family (Vu et al., 1991; Bohm et al., 1996; Ishihara et al., 1997; Xu et al., 1998; Boire et al., 2005; Vesey et al., 2007) as shown diagrammatically for KLK1 and KLK4 in Figure 1. These proteolytic agonists remove the PAR extracellular amino terminal pro-domain, unmasking a neo-epitope, termed the tethered ligand, that binds intramolecularly to induce intracellular signal transduction. Via these mechanisms, PARs function as plasma membrane sensors of extracellular and cell surface associated proteinases that regulate cellular responses such as proliferation, migration, inflammatory responses, thrombus formation and angiogenesis, and contribute extensively to homeostasis as well as to disease progression (Macfarlane et al., 2001; Adams et al., 2011).

Proteolytic activation of human PAR1, occurring on the carboxyl side of residue Arg^{41}, can be mediated by KLK1 (Gao et al., 2010a,b), KLK4 (Mize et al., 2008; Ramsay et al., 2008; Gratio et al., 2010), and KLK5, 6 and 14 (Oikonomopoulou et al., 2006). Similarly, PAR2 activation after Arg^{36} can occur through proteolysis by KLK2 (Mize et al., 2008), KLK4 (Mize et al., 2008; Ramsay et al., 2008), KLK5, KLK6 (Oikonomopoulou et al., 2006) and KLK14 (Oikonomopoulou et al., 2006; Gratio et al., 2010), while PAR4 activation by cleavage following Arg^{47} can be carried out by KLK14 (Oikonomopoulou et al., 2006). Consistently, another study demonstrated that while KLK6 is able to efficiently cleave a peptide spanning the PAR2 activation site, this proteinase could not cleave peptides spanning PAR1, PAR3 or PAR4 activation sites (Angelo et al., 2006). Interestingly, apart from thrombin no other PAR3 targeting proteinases have been identified (Ishihara et al., 1997). It is also important to note that KLKs can function as PAR antagonists by cleavage at sites other than the canonical activation site, leading to receptor inactivation or so-called 'disarming' (Adams et al., 2011). For example, KLK1 can disarm PAR1 (Gao et al., 2010a,b) and KLK14 can inactivate both PAR1 and PAR4 (Oikonomopoulou et al., 2006).

In terms of the functional consequences of KLK activation of PARs, several studies have demonstrated that these mechanisms regulate important cellular responses in both physiological and pathological settings. For example, KLK1 promotes keratinocyte proliferation and migration *in vitro* and in an *in vivo* rat skin wound healing model via a PAR1 dependent, BKR2 independent mechanism requiring downstream activation of PKC and Src resulting in increased matrix metalloproteinase activity (MMP) and regulation of EGFR signalling (Gao et al., 2010a). This group has also recently shown that KLK1, via PAR1, promotes migration and invasion of prostate cancer DU145 but not lung cancer A549 cells (Gao et al., 2010b). Signalling via this PAR and PAR2 induced by both KLK2 and KLK4 enhanced proliferation of prostate cancer DU145 cells (Mize et al., 2008). Interestingly, using murine whole splenocyte preparations and the human Jurkat T cell line it has been shown that KLK6 via PAR1 promotes pro-survival and inhibits pro-apoptotic mechanisms, leading to vigorous splenic T cell survival in the presence of the cell death inducing agents, camptothecin, dexamethasone, staurosporine and Fas-ligand (Scarisbrick et al., 2011). This KLK can also initiate intracellular signalling in neurons via PAR1 and in astrocytes through both PAR1 and PAR2 (Vandell et al., 2008) and has recently been reported to serve as a molecular trigger via PAR1 of processes involved in development of astrogliosis (Scarisbrick et al., 2012a). In addition, use of function blocking antibodies and pharmacological approaches demonstrated that KLK4 activates PAR1 but not PAR2 in colon cancer cells with PAR1 signalling promoting migration of these cells via ERK1/2 (Gratio et al., 2010). In contrast, KLK14 signals to HT29 colon cancer cells via PAR2 leading to receptor internalization and proliferation (Gratio et al., 2011). It is also noteworthy that KLK4, secreted by prostate cancer epithelia, can activate PAR1 in surrounding stroma to initiate release of the cytokine interleukin-6 that stimulates cancer cell proliferation in a paracrine loop (Wang et al., 2009).

Hepatocyte growth factor/Met receptor system

Hepatocyte growth factor (HGF) and its receptor MET are required for normal development and homeostasis while aberrant activation of this receptor system, via overexpression, mutation or dysfunctional ligand production and/or activation, contributes to malignant transformation and progression of a variety of cancers. In addition, these changes can often be associated with poor patient outcome and drug resistance (Trusolino et al., 2010). In its precursor state as pro-HGF, the presence of the pro-domain prevents activation of Met signalling. This pro-domain is released by the action of the Arg/Lys-specific serine proteinase HGF activator (HGFA), which itself must be converted from zymogen (pro-HGFA) to active enzyme by other serine proteinases such as thrombin (Mahtouk et al., 2010). In addition to this thrombin/HGFA/HGF Met receptor activating cascade, it appears that members of the KLK family can also initiate signalling via this receptor system through at least two levels as shown diagrammatically for KLK4 in Figure 1. The first level is via activation of pro-HGFA as it has recently been demonstrated that KLK4 and KLK5, but not KLK2 or KLK3, activate this zymogen at its canonical activation site Arg407 (Mukai et al., 2008). Importantly, KLK4 and KLK5 activated HGFA efficiently activated pro-HGF and induced cell scattering and invasion *in vitro* while these actions were strongly inhibited by HGFA inhibitor, a type 1, integral membrane Kunitz-type serine protease inhibitor that inhibits HGFA and other pro-HGF-activating proteases (Mukai et al., 2008). KLK-mediated regulation of this signalling cascade is also possible at the level of pro-HGF activation as it has been shown that KLK4 is able to activate urokinase-type plasminogen activator (Takayama et al., 2001) which is an efficient activator of pro-HGF (Naldini et al., 1992; Mars et al., 1993).

Plasminogen activator system

The plasminogen activator system is involved in cancer progression and metastasis through various processes, such as cell migration, adhesion and cell cycle regulation. Urokinase-type plasminogen activator (uPA) is a serine protease and its receptor uPAR (CD87) is a three domain (D1D2D3) glycoprotein tethered to the cell membrane with a GPI anchor (Huai et al., 2006). Expression of uPA-uPAR has been associated with prognosis in different cancers, although cleaved uPAR has stronger association with clinical outcome of patients than those with intact forms (Reuning et al., 2003; Rasch et al., 2008). For example, expression of uPAR lacking D1 predicted a poor prognosis in the patients with squamous cell lung cancer (Almasi et al., 2005), suggesting that the cleavage of this receptor has a role in cancer progression. KLK2, KLK4, and KLK8 can activate uPA (Frenette et al., 1997; Takayama et al., 2001; Rajapakse et al., 2005; Beaufort et al., 2006). Indeed, the first identified substrate of KLK4 was uPA (Takayama et al., 2001), which can also activate the metalloprotease (MMP) system via plasmin (Schmalfeldt et al., 2001; Smith

and Marshall, 2010), further linking these three proteolytic systems. Interestingly, a later study that examined the proteolytic effects of KLK4 at different concentrations (Beaufort et al., 2006) demonstrated that low levels of KLK4 cleaved uPA while higher KLK4 levels also had an effect on its receptor, uPAR (see Figure 1). This latter proteolytic action generates a soluble D1 fragment and D2D3 fragment still bound to the cell membrane. Beaufort et al. (2006) also showed that KLK4 cleaved uPAR on the membrane of U937 monocytes led to a reduced binding capacity to uPA. It is known that uPAR interacts with integrins and the ECM protein vitronectin (Smith and Marshall, 2010), and the function of the cell membrane-bound uPAR D2D3 domain is in binding to these adhesive proteins, promoting migration of cells and cancer progression. Interestingly, KLK7 is also suggested to play a role in the shedding of uPAR generating a soluble form of this receptor and reduced pancreatic cancer cell adhesion to vitronectin (Ramani and Haun, 2008b). Similar cell biology effects of the proteolytic action of KLK2, KLK4 and KLK8 on uPA-uPAR remain to be studied. Indeed, more in depth studies of the mechanisms involving these two protease systems are required to fully understand their interactive roles in human diseases.

EphB4 and its ligand ephrin-B2

The Eph receptor tyrosine kinases are the largest family of receptor tyrosine kinases with 14 members in two subfamilies designated A and B (Arvanitis and Davy, 2012). Eph receptors are involved in many cellular and developmental processes including development, homeostasis and disease (Bush and Soriano, 2012). Several Eph receptors are also over-expressed in cancer and novel anti-cancer strategies that target these proteins are in development (Nievergall et al., 2012; Boyd et al., 2014). EphB4, in particular, is commonly over-expressed in most epithelial cancers, including 66% of prostate cancers (PCa), 58% of breast cancers and 86% of ovarian cancers (Lee et al., 2005; Xia et al., 2005; Kumar et al., 2006; Kumar et al., 2007; Brantley-Sieders et al., 2011).

The ligands to Eph receptors are called ephrins and they too are divided into two subfamilies – ephrin-A and ephrin-B – based on sequence homologies and interactions with Eph receptors (Arvanitis and Davy, 2012). In contrast to most other ligands of receptor tyrosine kinases, ephrins are also membrane-bound on the outside of cells – ephrin-A ligands *via* glycosphosphatidylinositol (GPI) links and ephrin-B with a single transmembrane domain. Despite significant promiscuity between other Eph family members and the ephrin ligands, the single physiologically-relevant ligand of the EphB4 receptor is ephrin-B2 (Pasquale, 2004). In a similar manner to the classical trans-signalling pathways of Eph-ephrins, forward signalling, through EphB4, and reverse signalling, through ephrin-B2, requires heterotetramerisation of two receptors (on cell 1) and two ligands (on cell 2). This normal interaction between EphB4 and ephrin-B2, requires direct cell-cell contact and induces tumour suppression in the EphB4 expressing cell and stabilization of cell-cell adhesion and stimulation of angiogenesis through the ligand expressing cell (Chen et al., 2008; Pasquale, 2010). Disruption of cell-cell junctions or changes in the Eph-ephrin balance (through the overexpression of EphB4), both of which can occur in cancer cells, prevents or diminishes ligand-receptor interaction and allows the clustering of EphB4 and the use of ligand-independent signalling pathways that promote tumour formation. It has also been reported that Eph receptors and ephrins when co-expressed in neurons and cancer cells can engage in lateral cis associations, which lessen receptor activation through trans-signalling (Hornberger et al., 1999; Yin et al., 2004; Carvalho et al., 2006; Kao and Kania, 2011; Falivelli et al., 2013).

High affinity trans-signalling interactions between Eph receptors and ephrin ligands are terminated by two key mechanisms. In the first of these, the whole receptor-ligand complex is endocytosed into either cell *via* a mechanism that is dependent on Rac signalling and may include pinching off part of the plasma membrane of the opposite cell (Marston et al., 2003; Zimmer et al., 2003; Pitulescu and Adams, 2010). The second mechanism involves protease cleavage of the ligand or the EphR after formation of the receptor-ligand complex. Proteases linked to Eph receptor and ephrin proteins include RHBDL2, a rhomboid transmembrane serine protease that cleaves ephrin-B3, several matrix metalloproteinases and A Disintegrin and Metalloproteases (ADAMs) that have been reported to cleave both Eph receptors and ephrins, including ephrin-A2 and ephrin-B1 (Hattori et al., 2000; Pascall and Brown, 2004; Litterst et al., 2007; Tanaka et al., 2007; Lin et al., 2008; Inoue et al., 2009; Janes et al., 2009). Using indirect 'gain of function' approaches, EphB4 has been implicated as a potential substrate for ADAM-8, -9 and -17 (Janes et al., 2005; Guaiquil et al., 2009; Guaiquil et al., 2010; Mendelson et al., 2010; Weskamp et al., 2010).

More recently, we have identified EphB4 and its ephrin-B2 ligand as biochemical substrates of KLK4 (Lisle et al., 2013). An *in silico* approach was used to identify potential KLK4 cleavage sites through mapping the consensus KLK4 cleavage site to accessible epitopes on the surface

of proteins from protein databases, for which there were defined crystal structures. Ephrin-B2 was confirmed as a substrate of KLK4 by incubation of recombinant proteins in various molar ratios, and the primary cleavage site in ephrin-B2 confirmed using N-terminal sequencing as after R174 (NP_004084). Closely related kallikreins KLK3 (PSA), KLK2 and KLK4 can cleave ephrin-B2 and we have used a concentration of KLK peptidase at >4 μg/ml that is under the physiological range as the PSA concentration is 100–2000 μg/ml in normal male seminal fluid (Lovgren et al., 1999; Drabovich et al., 2011) while KLK2 is 2–20 μg/ml (Lovgren et al., 1999) and KLK4 is 0.2–202 μg/ml (Obiezu et al., 2005; Shaw and Diamandis, 2007).

Although EphB4 was not identified as a potential KLK4-substrate using the *in silico* approach (because a suitable crystal structure was not available), in parallel experiments recombinant EphB4 was also shown to be cleaved by recombinant KLK4 (Figure 2). The KLK4 cleavage site in EphB4 was identified by N-terminal sequencing and is in the extracellular domain between R508 and A509. Cleavage after R508 would release almost the complete extracellular domain of EphB4 and this was confirmed by addition of recombinant KLK4 to cells expressing surface EphB4 with subsequent identification of the cleaved ectodomain fragment of the predicted size via Western blot analysis. In addition, a protein band of the predicted size of the cleaved ectodomain was identified in cell lysates from EphB4+/KLK4+ prostate cancer cells, confirming that cleavage of the ectodomain can occur in a biological setting. Interestingly, this cleavage event appears to precede a second cleavage event catalysed by the presenilin-dependent protease γ-secretase, which releases the intracellular part of EphB4 from the membrane. This mechanism – ectodomain shedding followed by release of an intracellular fragment, is common to many receptor tyrosine kinases and in many cases the released fragments have important biological functions (Stephenson et al., 2012). The biological consequences of these two EphB4 cleavage events, and the functions of the released fragments, are currently being explored in clinical prostate cancer samples and cell lines.

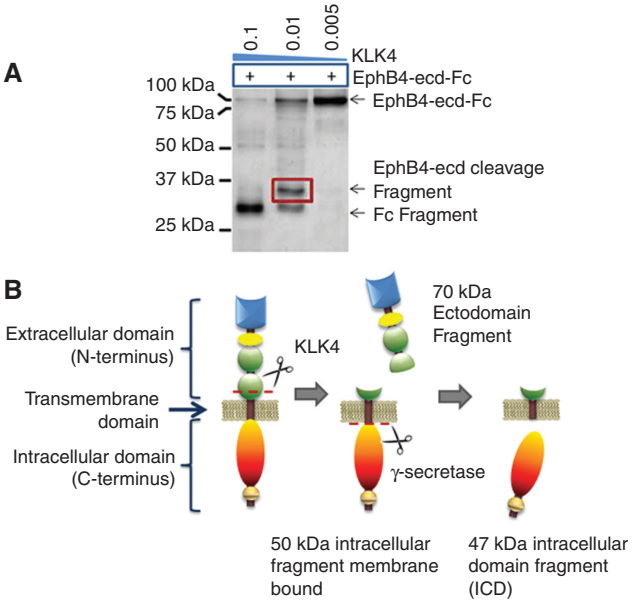

Figure 2 (A) Silver stained PAGE showing KLK4 cleavage of full length EphB4 extracellular domain tagged with IgG Fc tag (EphB4-ecd-Fc) at a ratio of active KLK4 to EphB4 of 1:10 (0.1), 1:100 (0.01) and 1:5,000 (0.005). The EphB4-ecd-Fc fragment generated by KLK4 activity is indicated by a red box. (B) Diagram showing KLK4 cleavage of human EphB4 releases an ectodomain (70 kDa) from the cell surface and leaves an intracellular membrane-tethered fragment (50 kDa). This cleavage is then followed by hydrolysis by γ-secretase releasing an intracellular domain (ICD) of 47 kDa.

CUB-domain containing protein 1 (CDCP1)

In the last few years the cell surface glycoprotein CUB-domain containing protein 1 (CDCP1) has emerged as a novel substrate of serine proteinases that have Arg/Lys trypsin-like substrate specificity (He et al., 2010), including, potentially, those members of the KLK family that cleave after these amino acids, such as KLKs 1, 2, 4–6, 8, 10–14. However, it is not known if CDCP1 can be cleaved by those with chymotryptic-like specificity, i.e., KLKs 3, 7 9, or with some cross chymotryptic substrate specificity. CDCP1, also known as SIMA135 (Hooper et al., 2003), gp140 (Brown et al., 2004), Trask (Bhatt et al., 2005) and CD318 (Wortmann et al., 2009), is an 836 amino acid single pass transmembrane glycoprotein that consists of extracellular and intracellular domains encompassing approximately 660 and 150 residues respectively. Serine proteinase-mediated cleavage occurs within the extracellular domain at adjacent sites, Arg368 and Lys369, to initiate Src-mediated phosphorylation of CDCP1 at tyrosine (Y) 734, Y743 and Y762 and binding of phospho(p)-Src and another kinase p-PKCδ (He et al., 2010). Recently it has been shown that serine proteinase-mediated cleavage of CDCP1 promotes the survival of disseminating cancer cells as they escape from blood vessels. Increased cancer cell dissemination occurs via a p-Src/p-PKCδ mechansism that initiates p-Akt pro-survival and anti-apoptotic pathways (Casar et al., 2012). CDCP1 is up-regulated in cancers of the lung, kidney, ovary and pancreas where its elevated

expression is associated with poor patient outcomes (Awakura et al., 2008; Ikeda et al., 2009; Miyazawa et al., 2010; Razorenova et al., 2011; Dong et al., 2012). As CDCP1 is widely expressed in mammalian epithelia including these cancers of epithelial origin where elevated KLK expression is also commonly observed (Lawrence et al., 2010), we were interested to see if members of this family are able to cleave CDCP1. In our hands, low levels of KLK4 were able to induce CDCP1 cleavage within 6 h, generating a 70 kDa fragment (Figure 3A), with the schematic shown in Figure 3B, although the function of this cleaved product remains to be investigated. These data suggest that in settings where active KLK4 or other Arg/Lys-specific KLKs are present, CDCP1 may act as a plasma membrane substrate to transduce signals across the cell surface including that promoting cell survival.

CDCP1 is known to be cleaved by matriptase and trypsin, which also cleave PAR2 (Brown et al., 2004; He et al., 2010) but it is thought that the endogenous protease is plasmin as it was found to be necessary and sufficient to cleave CDCP1 *in vivo* and plasminogen knockout mice lacked cleavage of CDCP1 in lung tissue (Casar et al., 2012). Increasingly, the expression of protease activated receptors and other proteolytically-activated proteins such as CDCP1 is becoming more clinically significant as potential markers of disease progression and as molecular targets. For example up-regulation and aberrant expression of both CDCP1 and uPAR are correlated with a poorer prognosis and can be indicative of disease progression in some cancer types (Awakura et al., 2008; Ikeda et al., 2009; Ma and Tao, 2012). Furthermore, as plasmin is generated from plasminogen by uPA it is possible that CDCP1 and other protease activated receptors could be implicated in the same pathway, contributing to dissemination and metastasis of invading tumour cells and therefore could represent multiple targets within a common pathway. As we noted for the HGF/Met activation and uPA/uPAR cascades, it appears likely that KLKs will also be able to contribute to activation of CDCP1 signalling at multiple levels as it has recently been shown that plasmin is the crucial serine protease executing *in vivo* cleavage of this receptor during cancer cell dissemination in mice (Casar et al., 2012).

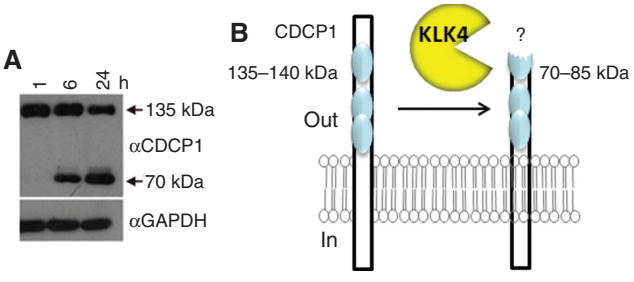

Figure 3 (A) Anti-CDCP1 and -GAPDH Western blotting of lysates from HeLa-CDCP1 cells incubated with KLK4 (50 nM) at 37°C for 1, 6 and 24 h. Full-length (135 kDa) and cleaved (70 kDa) CDCP1 are indicated. (B) Schematic showing that KLK4 cleavage of full length CDCP1 generates a 70 kDa form.

Other cell membrane-bound proteins as KLK substrates

In addition to the above receptor systems, there are a range of other associated cell surface proteins that are cleaved by members of the KLK family. For example, KLK6 can hydrolyse amyloid precursor protein (APP), which is localized in senile plaques and neurofilament tangles in the brain of Alzheimer's disease (AD) patients and is important in the deposition of amyloid plaques during progression of this disease (Little et al., 1997; Ogawa et al., 2000; Magklara et al., 2003). Also, KLK6 is able to cleave myelin basic protein (MBP) in oligodendrocytes and Schwann cells in the central nerve system, which is related to demyelinating diseases such as multiple sclerosis (Scarisbrick et al., 2012b). Although KLK5 and KLK7 have been localised in numerous tissues their function has been extensively elucidated in keratinization, stratum corneum formation, turnover and desquamation of the skin. This occurs via the hydrolysis and degradation of cell membrane-bound adhesion glycoproteins, such as corneodesmosin, desmocollin 1 and plakoglobin (Caubet et al., 2004).

The cadherin proteins, desmogleins (DSGs), are key factors in desmosome formation and cell integrity in particular in skin (Amagai and Stanley, 2012), and loss of them has also been associated with cancer progression (Thiery and Sleeman, 2006). DSG2 is a substrate of KLK7 and transfection of this peptidase significantly upregulated levels of soluble DSG2 fragments in culture media from pancreatic cancer BxPC-3 cells (Ramani et al., 2008c). Interestingly, using siRNA knockdown and protease inhibition approaches, cleavage of DSG1 by KLK5 was induced in oral squamous cancer SCC25 cells where decreased aggregation of these cells was seen (Jiang et al., 2011). It is known that the cell-cell contact protein, E-cadherin, plays a critical role in cell integrity and loss or degradation of this molecule has been associated with cancer progression (Thiery and Sleeman, 2006). Of note, adding KLK7 into pancreatic cancer BxPC-3 cells generated more soluble E-cadherin fragments while the aggregation of these cells significantly decreased, but invasion increased (Johnson et al., 2007). These data suggest a role of these

KLK peptidases in the progression of cancer by hydrolysis of cell adhesion proteins.

Meprins are members of the astacin metalloprotease family and have α and β subunits originally identified on the apical brush or plasma membrane of epithelial cells (Bond et al., 2005). Their unique localization and proteomic activity have been associated with trafficking of host cells such as, macrophage or metastatic cancer cells (Bond et al., 2005). A recent study using a terminal amine isotopic labeling of substrates (TAILS) approach identified that pro-KLK7 is a substrate of meprin α and β in the conditioned media from keratinocyte HaCaT cells (Jefferson et al., 2012) supporting their function in skin epithelial cells. Interestingly, both meprin α and β subunits were localized in the plasma membrane of HaCaT keratinocytes and pro-meprin β was cleaved by KLK4 (Becker-Pauly et al., 2007; Ohler et al., 2010), further suggesting a complex interaction between these two protease families.

Conclusion

Here we have summarised the current knowledge on the hydrolysis of cell membrane receptors/proteins by KLK1 and other KLK peptidases (Table 1) and their initiation of downstream signalling pathways. Although there is a diversity of tissue specificity and expression of these proteases, we have identified a strong association between the classical membrane-bound receptor activation function of KLK1 and similar attributes of other more recently discovered members of the KLK family. In addition, it is notable that there is increasing evidence of complex proteolytic cascades between the KLKs, uPA/uPAR and metalloproteinase families. A better understanding of these interactive cascades and downstream cellular signalling pathways initiated by these peptidases will help to fully elucidate the biological functions and pathophysiological role of KLKs *in vivo*.

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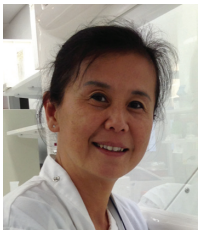

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